CLINICAL STUDY SYNOPSIS

Title of Study: A randomized, single-blind, parallel-group, multi-center comparative study to assess the efficacy and safety of an application of Fybex® (fibrin sealant) to the operative field, for the reduction of post-operative blood loss, when added to standard intra-operative hemostatic procedures and compared to standard intra-operative hemostatic procedures alone, in adult patients undergoing total knee arthroplasty

Protocol Number: Fybex02 / PAREXEL 71786

Study Period: **Date of first enrollment:** 27 Feb 2006 **Date of last completed:** 04 Dec 2006	**Phase of Development:** III

Investigator(s) and Study Center(s): Nine centers (seven in Poland, two in the United Kingdom [UK]) were initiated for this study with ten investigators (seven in Poland, three in the UK). Due to difficulties in recruitment, one investigator did not recruit any patients. For a list of these study centers and investigators, refer to Section 16.1.4.

Publication(s): None.

Objectives:

Primary objective

To investigate the efficacy of Fybex® in reducing post-operative blood loss in patients undergoing total knee arthroplasty (TKA).

Secondary objectives

To investigate the hemostatic effect and safety, including local tolerance and viral safety, of Fybex plus standard intra-operative hemostatic procedures compared to standard intra-operative hemostatic procedures alone.

Study Design: This was a randomized, single-blind, multi-center study to compare the efficacy and safety of an intra-operative application of Fybex plus standard intra-operative hemostatic procedures to reduce post-operative blood loss compared with standard intra-operative hemostatic procedures alone in patients undergoing TKA. There was a maximum of nine study visits: screening (two to 6 weeks prior to surgery), Day -1, Day 0 (TKA surgery), Days 1, 2, 3, 4, 7 and a follow-up visit 3 months after surgery.

Number of Patients (planned and analyzed):

Planned: Approximately 96 patients were to be enrolled in the study. Before randomization, 30 patients were to be treated with the study medication according to the protocol in order for the investigator to become familiar with the application method. A total of 66 patients were to be randomized, 33 to each treatment group.

Analyzed: A total of 95 patients were included in the full safety analysis set. Of these, 19 were included in the familiarization group before randomization, and 76 were randomized (40 patients were randomized to receive Fybex and 36 patients to standard operation procedures).

Diagnosis and Main Criteria for Inclusion: Patients aged ≥ 18 years, able to provide written informed consent and who were scheduled to undergo elective primary unilateral TKA OR scheduled to undergo elective primary bilateral sequential TKA (if it was the first knee in a patient whose second knee was to be replaced at least 3 months later, or the second knee in a patient whose first knee had been replaced at least 3 months earlier), were enrolled in the study.

Test Product, Dose and Mode of Administration, and Batch Numbers:

Product:	Fybex
Dose:	5 milliliter (ml) user kit, producing 10 ml of sealant
Mode of administration:	Intra-operatively, sprayed onto open tissue
Batch numbers:	FKCN7142, FKCN7143, FKCN7270, FKCN7244

Reference Therapy, Dose and Mode of Administration, and Lot Number(s): None.

Duration of Treatment: There was a maximum of nine study visits: a screening visit (2 to 6 weeks prior to surgery) to assess patient eligibility, Day -1 (further eligibility testing; this visit could be held on Day 0 in accordance with the standard local procedures regarding admission of patients for surgery), Day 0 (the day of TKA surgery), Days 1, 2, 3, 4, and 7 and a follow-up visit 3 months after surgery.

Criteria for Evaluation:

Efficacy:

Primary efficacy

- Post-operative blood loss to 48 hours post-surgery

Secondary efficacy

- Post-operative blood loss to removal of drain
- Total measured peri-operative blood loss (intra- plus post-operative blood loss) to 48 hours post-surgery
- Post-operative decrease in hemoglobin (Hb) and hematocrit (HCT) at 24 hours (± 4 hours) post-surgery or, for patients who received blood transfusions prior to 20 hours post-operatively, post-operative decrease in Hb and HCT up to the pre-transfusion blood sample
- Blood transfusion requirements up to 48 hours post-surgery (whether required and how many units required).

Safety:

- Type, frequency and severity of adverse events (AEs)
- Viral safety
- Library assay (blood samples were only investigated at the end of the study for thrombogenicity testing, if there was an alteration in a patient's coagulation tests)
- Changes in serum biochemistry and/or hematology
- Physical examination
- Vital signs.

Statistical Methods:

The analysis of the primary efficacy endpoint was based on the intention-to-treat (ITT) set. There was a secondary analysis based upon the per protocol (PP) set, to assess the sensitivity of the analysis to the choice of analysis population. The analysis of all other endpoints was based on the ITT set only.

Demographic data, medical history, concomitant medication, specific surgical approach, type of anesthesia, type of drainage system and type of prosthesis inserted are summarized by means of descriptive statistics or frequency tables, stratified by treatment. All safety analyses were based upon the full safety analysis set (familiarization patients plus randomized patients).

Efficacy:

Corrected analyses were required for the primary and secondary endpoints related to blood loss due to an error in the formula used to calculate the amount of blood loss in the post-operative period. The primary and secondary efficacy parameters and endpoints were not changed from those stated in the protocol; only the method of calculation. Therefore, data generated from both the incorrect formula and the corrected formula are presented in this report.

In brief, the formula in the protocol advised subtracting the (known) volume of sodium citrate used to prime the drainage bag (which was provided to prevent drainage fluid from clotting) from the total volume of fluid measured in the bag when it was changed. However, the calculation of the total blood loss relies upon relating the concentration of Hb in the bag to the patient's recent or average Hb concentration (Hb_{AV}) from a full blood count. The Hb content of the bag is derived from the assayed concentration in an aliquot of mixed fluid from the bag and the total volume of fluid contained in the bag. Use of the total volume in the calculations is correct as the Hb is distributed across the total volume of drainage fluid and sodium citrate. Therefore, the formula in the protocol should not have advised subtraction of the volume of citrate from the volume of fluid in the bag.

For the primary efficacy endpoint, post-operative blood loss during the 48 hours following surgery was analyzed using an analysis of covariance (ANCOVA) model, using log transformed blood loss data. Treatment and investigator were included in the model.

For the secondary efficacy endpoints, post-operative blood loss to removal of drain, total measured peri-operative blood loss to 48 hours post-surgery, total measured peri-operative blood loss to removal of drain, and post-operative decrease in Hb and HCT were analyzed using similar techniques as for the primary efficacy parameter.

The proportion of patients who received a transfusion was analyzed by treatment using Fisher's Exact test. The total number of units of blood or blood products containing red blood cells transfused was summed across product types. The median number of units of blood or blood products containing red blood cells was calculated using the Hodges-Lehmann estimator and was presented along with the corresponding 95% confidence interval (CI) and p-value from the exact permutation test.

Safety:

The duration of the operation was summarized by treatment group using summary statistics, and a by-patient listing of the duration of operation was presented. A by-patient listing of duration of anesthesia was presented. The number and percentage of patients who received revision surgery were to be summarized by treatment group. No patients received revision surgery during this study.

All AEs were followed either to resolution, until an underlying condition had been diagnosed, until the patient's condition had stabilized or for a period of 28 days following the last study visit. Any new or unresolved AEs noted at the last study visit were followed up for a further 28 days unless they were resolved, the condition diagnosed or the condition stabilized.

An overall summary of AEs was provided. AEs were also summarized by system organ class (SOC) and preferred term, severity and causality. A by-patient listing of all AEs was provided. The number and percentage of patients who reported an SAE and the number of SAEs by treatment group, SOC and preferred term were presented. SAEs were also summarized by causality. By-patient listings were provided for all deaths that occurred during the study, all SAEs, and all AEs that lead to withdrawal from the study.

Central laboratory hematology and serum biochemistry were summarized using summary statistics for the absolute values and change from baseline values at all available visits during the study. Shift tables for the change from baseline to each planned post-baseline visit were also provided.

For virology, the number and percentage of patients with non reactive or reactive results were presented by visit, and shift tables of the change from baseline to each planned post-baseline visit were presented. Library assays were to be measured at follow-up for thrombogenicity testing, only if there was an alteration in a patient's coagulation tests, if there was a clinically significant change in a patient's coagulation test. No patients had a library assay performed during this study. By-patient listings of all central and local hematology, serum biochemistry, virology and library assay laboratory data were provided. Both central and local laboratory coagulation assessments were summarized for the absolute values at all available visits during the study. By-patient listings of coagulation assessments were provided. Vital signs were summarized for absolute values and change from baseline at all available visits, and by-patient listings provided. The number and percentage of patients with abnormal physical examinations were presented by body system at screening and follow-up and a by-patient listing of physical examination data including descriptions of the abnormalities was provided.

Efficacy Results:

The primary objective of this study was to investigate the efficacy of Fybex in reducing post-operative blood loss in patients undergoing TKA. Corrected analyses were required for the primary and secondary endpoints related to blood loss due to an error in the formula used to calculate the amount of blood lost in the post-operative period (Sections 9.5.1.3.1 and 9.7.1.7.1).

In the ITT set, the LSMeans for post-operative blood loss to 48 hours following surgery (corrected analysis) were 617.95 ml for the randomized Fybex group, and 532.70 ml for the randomized standard operation group (relative blood loss Fybex/standard operation: 1.16). In the PP set, the relative blood loss Fybex/standard operation was 1.25. The differences between treatment groups in post-operative blood loss to 48 hours following surgery in the ITT and PP sets were not statistically significant ($p=0.310$ and $p=0.204$, respectively). Original analysis of the primary objective also showed no statistical significance in the ITT or PP sets ($p=0.242$ and $p=0.163$, respectively).

However, differences in post-operative blood loss were noted between the centers when comparing post-operative blood loss to 48 hours following surgery by center. The comparison between randomized Fybex patients versus standard operation patients showed a reduction in the Fybex group of >15% for two investigators (205 and 208, using an antero and antero-medial operative approach, respectively), an increase of > 5% for investigator 210 (using an antero approach), an increase > 20% for investigator 206 (using an antero-medial approach) and increases of > 40% and > 70% for investigators 207 and 209, respectively, both using an antero operative approach. Similar results were observed in the original analysis of differences in post-operative blood loss to 48 hours following surgery between centers. Differences in the application of Fybex by the different investigators could explain the variations recorded in blood loss.

With respect to the secondary endpoints, corrected analysis of the differences observed between treatment groups of the ITT set in post-operative blood loss to removal of drain were not statistically significant (p=0.306). Initial analysis of post-operative blood loss to removal of drain also showed no statistical significance (p=0.240). No statistically significant differences were observed for corrected analyses between treatment groups in total peri-operative blood loss to 48 hours post surgery (p=0.189), or total peri-operative blood loss to removal of the drain (p=0.186). Original analyses of these secondary endpoints were also found to have no statistical significance (p=0.146 and p=0.144, respectively). Furthermore, secondary endpoints, post-operative decrease in Hb at 24 hours post surgery (p=0.985), post-operative decrease in HCT at 24 hours post surgery (p=0.899), blood transfusion requirements up to 48 hours post surgery (p=0.821), or total number of units of blood or blood products containing red cells used in blood transfusions (p=0.958) also lacked statistical significance.

The corrected exploratory analyses of post-operative blood loss during the 48 hours following surgery including weight as a covariate and gender as a factor one at a time showed no statistically significant differences between treatment groups in post-operative blood loss during the 48 hours following surgery (p=0.350 and p=0.315, including weight or gender, respectively). Similar results were observed for original exploratory analyses of post-operative blood loss during the 48 hours following surgery including weight and gender separately (p=0.280 and p=0.247, respectively).

Safety Results:

One death was reported during this study in the randomized standard operation group: patient 05/206/K665 died due to SAEs of *cardiac arrest* and *pulmonary embolism*. Seven patients experienced 12 SAEs: four patients receiving Fybex (two randomized and two familiarization patients), and three patients in the standard operation group. Of the SAEs reported by patients receiving Fybex (randomized and familiarization groups), two of the events were severe in severity and only one event was considered possibly related to Fybex: patient 01/209/K751 experienced a severe SAE with the preferred term *hepatitis C*. The risk of transmission of blood-borne pathogens associated with blood products such as Fybex, including hepatitis C, is described in the Investigator Brochure, and a relationship to Fybex could therefore be suspected for this patient. However, further PCR and antibody tests confirmed that the initial HCV PCR test at follow-up was a false positive and that the patient was HCV negative at the final study visit. Of the seven SAEs reported in the randomized standard operation group, six were severe in severity and two of these SAEs resulted in the death of one patient.

A total of 319 AEs were reported during this study by the 95 patients in the full safety analysis set. The most frequently reported AEs were events with preferred terms *pain* (52/95 [54.7%] patients), *procedural pain* (43/95 [45.3%] patients) and *pyrexia* (42/95 [44.2%] patients). No notable differences were reported between treatment groups for any of the more frequently reported AEs.

Six (6.3%) patients experienced ten severe AEs. One patient in the randomized Fybex group experienced one severe SAE with the preferred term *hepatitis C*, which was confirmed to be a false positive after further PCR and antibody tests. In the randomized standard operation group, two patients experienced six severe SAEs: one patient experienced arrhythmia, *hyperglycaemia, depressed level of consciousness* and *hypotension*; the other patient experienced *cardiac arrest* and *pulmonary embolism*, which resulted in death. Three patients in the familiarization group experienced three severe AEs of *deep vein thrombosis* (SAE), *hypotension* and *atrial fibrillation*.

All patients experienced AEs possibly or probably related to surgery/anesthesia and 7/59 (7.4%) patients receiving Fybex experienced AEs considered to be possibly related to Fybex: five patients (two randomized and three familiarization patients) experienced *pyrexia,* one familiarization patient experienced *oedema peripheral* and *pain in extremity,* and one patient in the randomized Fybex group experienced *hepatitis C* (which was a false positive, as described above). Hypersensitivity or allergic reactions, including *oedema*, are potential risks with fibrin sealant/haemostatic products. *Pyrexia* and *pain in extremity* were not listed as expected in the Investigator Brochure, but could be expected as a result of TKA.

There were no notable differences between treatment groups in hematology results. The majority of patients had low Hb and HCT values from Days 1 to 7 that returned within normal ranges for the majority of patients at follow-up. The results were similar for red blood cell counts. Most patients had normal

platelet values from Days 1 to 4, a large proportion had high values on Day 7 and values returned within the normal range for the majority of patients at follow-up. Over one third of patients had an increase from normal baseline to high white blood cell counts on Day 1. White blood cell counts gradually returned to normal from Day 3 and were within the normal range for the majority of patients at follow-up.

Glucose values tended to increase in all treatment groups on Day 1. This increase was more marked in the familiarization patients compared to the randomized (Fybex and standard operation) patients. Glucose values gradually returned to normal for all treatment groups and were normal for the majority of patients at follow-up. There were no notable differences between treatment groups in the remaining biochemistry results. The majority of patients had normal values for the remaining biochemistry parameters throughout the study.

No notable differences were observed between treatment groups in virology parameters. The majority of patients were reactive to HAV antibody, and all were negative to HAV antibody IgM, at screening and follow-up. All but one patient were negative for HIV antibody, at screening and follow-up. This patient was in the randomized Fybex group. At screening, all patients were negative for HCV antibody; one patient in the randomized Fybex group was positive for HCV when tested by PCR at the follow-up visit, but it was later shown that this result was a false positive. There were no notable differences between treatment groups in coagulation tests.

There were sporadic individual clinically significant abnormalities observed in hematology and urinalysis, which returned to normal at the follow-up visit. With regards to virology, one patient in the randomized Fybex group experienced a severe SAE with the preferred term *hepatitis C* during the study, which was a false positive, as described above; the patient was HCV negative at the final study visit.

Conclusions:

The differences between treatment groups for the corrected primary endpoint (post-operative blood loss to 48 hours following surgery) in the ITT (relative blood loss Fybex/standard operation: 1.16) and PP (relative blood loss Fybex/standard operation: 1.25) sets were not statistically significant (p=0.365 and p=0.304, respectively). Similar results were observed in the original analysis.

Differences in post-operative blood loss were noted between the centers when comparing post-operative blood loss to 48 hours following surgery by investigator, for the corrected and original analysis. Differences in the application of Fybex by the different investigators could explain the variations recorded in blood loss. One hypothesis is that the area on which the spray is applied makes a difference to Fybex effectiveness on blood loss. It is of interest to note that variability between centers was previously reported in the literature. A previously published phase III study comparing the use of a different fibrin sealant combined with standard operating procedures to standard operating procedures alone showed that, for five surgeons, a reverse trend was observed, i.e. a greater volume of blood lost in the group receiving fibrin sealant.

With respect to the secondary endpoints (corrected and original analyses), the differences observed between treatment groups of the ITT set were not statistically significant, and the exploratory analyses (corrected and original) performed showed no statistically significant differences between treatment groups. In conclusion the study did not provide any evidence that Fybex produced a statistically significant reduction in blood loss in patients undergoing TKA.

Fybex was shown to be a safe product, with no notable differences observed between treatment groups for safety parameters.

Date of Report: 11 May 2007

Sponsor:

Medical Director Dr C.H. Dash

Signature

25 June 2007

Date